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Its metabolite (desbutylhalofantrine) was less effective than halofantrine in curing Vietnam Oak Knoll P. falciparum infections. MSP-1, a DNA erythrocytic vaccine did not prevent virulent infections after Oak-Knoll challenge. Sterile immunity in Aotus has been achieved by repeated Oak Knoll challenges. A fourth immunization with a DNA pre-erythrocytic vaccine has produced high antibody levels. To date, sporozoites of the 3D7 clone of P. falciparum have not produced patent infections. Vaccination with Interleukin-12 did not yield gamma interferon in Aotus. A fourth (i.d.) vaccination with DNA Py CSP vaccine generated highly significant antibody titers.

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Richard N. Rossan 21 March 1996

21 March/96

PI - Signature Date

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INTRODUCTION

The essence of the problem addressed in this report is: 1) to evaluate the potential antimalarial activity of drugs in the pre-clinical model of Aotus lemurinus lemurinus (Panamanian night monkey) experimentally infected with Plasmodium falciparum or P. vivax, and 2) to use this model to test recombinant DNA malaria vaccines. Drug evaluation studies were supported by the U. S. Army, while the vaccine studies received support from the U. S. Navy Malaria program. Studies with this model were initiated in 1976 at Gorgas Memorial Laboratory, Panama. Due to the drug resistance exhibited by the highly pathogenic P. falciparum parasites in Asia, Africa, and Latin America, it is essential that new drugs be evaluated in the preclinical Aotus model for their potential usefulness against human infections.

Initially, antimalarial drug studies used the Colombian Aotus as the experimental host (1, 2). In the mid 1970's embargoes imposed by South American countries on the exportation of monkeys seriously restricted the use of Aotus for biomedical research in the United States. Panamanian Aotus were available at Gorgas Memorial Laboratory, Panama, and the project transferred here in 1976. Diverse avenues of research have been pursued in attempts to identify effective new antimalarial drugs. Three strains of P. falciparum, Vietnam Smith, Uganda Palo Alto, and Vietnam Oak Knoll, had been adapted to Panamanian Aotus. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents. The course of untreated infections in Panamanian Aotus has been characterized and compared with that in Aotus of Colombia (3). Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for the evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more P. falciparum strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of P. falciparum (4,5). Desferrioxamine, an iron-specific- chelating agent, was shown to suppress parasitemias of the virulent Uganda Palo Alto strain of P. falciparum (6). The in vitro activity of

two halogenated histidine analogs was not confirmed by evaluation against P. falciparum infections in owl monkeys (7).

Chloroquine-resistance of P. falciparum represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in P. falciparum, in vitro, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine (8). Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria (9). Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of in vitro reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in Aotus infected with the chloroquine-resistant Vietnam Smith strain of P. falciparum. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin , a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo (10). Parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment (11).

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant P. falciparum strain in Aotus.

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against P. falciparum infections in Aotus. The method of

approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of P. falciparum. If successful, it will establish for the first time that DNA vaccines can protect non-human primates, a critical step forward using DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one or more points: anti-sporozoite antibodies that prevent invasion of hepatocytes; cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes; antimerozoite antibodies that prevent invasion of erythrocytes; antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses; antibodies that attack infected erythrocytes; cytokines that kill parasites within erythrocytes; and, anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccine have shown that the Panamanian Aotus P. falciparum model to be suitable for this purpose. (12, 13, 14)

BODY

I. Experimental Methods

The first intent of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of Aotus experimentally infected with P. falciparum (or P. vivax). Specifically, the vertebrate host is Aotus lemurinus lemurinus, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in Aotus. The Vietnam Smith/RE strain of P. falciparum was adapted to Aotus of Colombian origin in 1971 (1) and in Panamanian Aotus in 1976. (3). The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian Aotus (3). This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine (2).

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated Aotus was diluted appropriately in chilled saline (0.85%), such that each milliliter contained 5,000,000

parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. (15)

Blood films from untreated Aotus, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8° C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

The second intent of this project is to ultimately evaluate recombinant vaccines against the blood and sporozoite stages of P. falciparum and against the blood stages of P. vivax in the Panamanian Aotus model. Prior to actual anti-parasitic experiments various routes of administration of a candidate vaccine must be tried so as to produce significant antibody levels. These trials will be detailed in the appropriate sections, as will other experiments associated with the Navy Malaria program.

II. Results

A. Toxicity of WR 227825AD (BH 35430)

Toxicity of this pyrroloquinazoline, as given in the previous annual report showed that dosages of 1.0 and 4.0 mg/kg, twice daily, for 3 days, led to the monkey's death. When the dose of the drug was reduced to 0.1 mg/kg, once daily for 3 days, or co-administered with WR 139004AC (BK 64208), 1.0 mg/kg, folinic acid, both animals survived without significant weight loss.

Since the results of co-administration of folinic acid were inconclusive, a further study was initiated, using four Aotus cured of P. falciparum and P. vivax infections. Two animals were administered WR 227825 at a dose of 1.0 mg/kg, orally, once daily for 3 days, and 2 animals received the drug plus folinic acid, 1.0 mg/kg, once daily for 3 days. The two monkeys, with WR 227825 alone, survived, experiencing a 3% body weight loss on day 12 post treatment and a 20% body weight loss on day 6 post treatment, respectively. Both animals that received the combination regimen died, one on day 5 post treatment, 15% bodyweight loss, with gross findings of nephritis and hepatic lesions; the second monkey succumbed on day 7, post treatment, 17% body-weight loss, and showing pneumonic foci.

B. WR 171669AU (BM 01792), halofantrine
WR 178460AC (BM 08577), desbutylhalofantrine

Halofantrine, a 9-phenanthrenemethanol, was shown by Schmidt (16) cure Vietnam-Oak Knoll infections in Colombian Aotus when administered at a dose of 20.0 mg/kg (x 7 days). The drug was less effective against Smith strain infections in Aotus of Panamanian origin. Clinical trials with halofantrine indicate its tendency to cause prolongation of QT intervals, as well as to adversely affect food intake, producing thiamine deficiency, and involve a risk of drug-drug interactions.

Desbutylhalofantrine, the metabolite of halofantrine, not only has less propensity to prolong QT intervals, but has similar or greater antimalarial activity both in vitro and in the mouse model. An experiment was designed to: 1) compare the blood schizontocidal of the two drugs

against Vietnam-Oak Knoll strain infections in Panamanian Aotus, and 2) obtain blood samples for pharmacokinetic studies, using an HPLC assay.

A total of 15 Panamanian Aotus was inoculated with the Vietnam-Oak Knoll strain of P. falciparum, 6 to be treated with halofantrine, 6 to be treated with its metabolite, and 3 controls. Detailed parasite responses to WR 171669 (halofantrine) are presented in Table 1, and summarized in Table 2. Doses of 5.0 and 20.0 mg/kg cleared primary parasitemias in all monkeys, while infection cure was achieved only after one or more retreatments at higher doses.

In contrast, WR 178460 (desbutylhalofantrine), 5.0 mg/kg did not clear parasitemias in 3 of 3 animals (Tables 3 and 4), but did at a dose of 20.0 mg/kg. Again, infection cure was seen after one or more retreatments, except that no cure in each of two animals following 90.0 mg/kg.

The data in Table 5 present an overall summary of the two drugs.

C. Establishment of a Plasmodium falciparum (Santa Lucia strain) sporozoite model.

In order to test a projected plasmid DNA vaccine against Plasmodium falciparum sporozoites, it is necessary to establish a Panamanian Aotus model. The Santa Lucia strain of P. falciparum was selected because of extensive use of this parasite by Dr. W. Collins, CDC, Atlanta, GA, who has consistently obtained infections induced by sporozoites, albeit in splenectomized Aotus of South American origin.

In February, 1995, 12 Aotus were each inoculated intravenously with 20,000 sporozoites of the Santa Lucia strain and divided into three groups of four animals each as follows: Group 1 - splenectomized prior to inoculation, Group 2 - splenectomized on day 7 postinoculation, and Group 3 - splenectomized on day 37 postinoculation.

The data, summarized in Table 6, indicate that patent infections were established in a total of 7 (58%) Aotus, 2 in Group 1, 3 in Group 2, and 2 in Group 3. Pre-patent periods ranged from 21 to 39 days post-inoculation, with a mean of 25.5% days and a standard deviation of 6.5 days. The appearance of parasites for one day in Aotus 12741, on day 29, was excluded from this calculation. It should be noted that parasites in Aotus 12347, appeared in blood films prior to splenectomy. A mean maximum

parasitemia (x 10^3) of 423 per cmm with a standard deviation of 169 per cmm developed in splenectomized monkeys.

D. Assessment of an erythrocytic DNA vaccine - MSP-1

For the first trial of an erythrocytic vaccine in this model, a total of 9 malaria naive Aotus were divided into three groups of three animals each and vaccinated as follows:

Group 1 - gd P*f* MSP1-19	500µg ID
Group 2 - gd P*f*s 25	500µg ID
Group 3 - Vi P*f*s 25	500µg ID

Group 1 vaccine is targeted against merozoite surface protein, the Group 2 vaccine against sexual stages, and Group 3, also targeted against the sexual stages but with a different vector than in Group 2. Animals received three vaccinations at three-week intervals, and on day 14 after the last vaccination, each monkey was inoculated intravenously with 10,000 parasites of the Vietnam-Oak Knoll strain of P. falciparum.

That no protection was afforded by the vaccine is indicated by patent, virulent infections beginning on day 8 post challenge in all monkeys. Infections were cured by mefloquine treatment (40.0 mg/kg x 3 days). These monkeys were then incorporated into the Vietnam-Oak Knoll rechallenge study.

E. Induction of immunity by repeated challenge with the FVO strain of P. falciparum

Of the various P. falciparum strains adapted to non-human primates, the FVO (Vietnam-Oak Knoll) strain would be useful for vaccine studies as only 25-30% of infected Panamanian Aotus self-cure (3). The remainder of the infected animals require curative drug treatment or death will ensue. When evaluating a vaccine, the higher the proportion of self-cure, the greater the number of animals needed in each experimental group to assure that the animals are protected by the vaccine and not self-curing.

To compare the efficacy of an "artificial" vaccine with protection afforded by acquired immunity, an experiment was initiated to induce immunity by repeated trophozoite challenge. Initial results were given in the previous report. Briefly, malaria naive Panamanian Aotus were inoculated with 10^4 parasites of the FVO strain, the parasitemia monitored daily by blood film examination, and the infection cured with mefloquine (40.0 mg/kg, oral, x 3 days) when parasitemia approximated 800,000 per cmm. About 4 to 6 weeks after infection cure, the animals will be rechallenged with parasites from a donor monkey whose infection was initiated by cryopreserved parasites. Donor animals, cured of infection, were recycled into the challenge group. Challenges will be repeated until the monkeys demonstrate complete immunity.

The current results summarized in Table 7 indicate that sterile community has been induced in four monkeys following 2, 3 or 4 rechallenges. Sera will be obtained twice from the 14 animals, one month apart, and then re-challenged with FVO parasites. Following this homologous rechallenge, a heterologous challenge is planned with a plasmodium strain yet to be determined.

F. Assessment of a pre-erythrocytic DNA vaccine - CSP/SSP2/EXP1

This study, initiated in September 1995, consisted of 32 malaria naive, laboratory born Panamanian Aotus, divided into four groups of eight animals each. Group 1 animals were vaccinated intradermally with CSP/SSP2/EXP-1, circum sporozoite surface protein exported protein; the animals in Group 2 received the same vaccine, administered intramuscularly; Group 3 subjects were vaccinated intramuscularly only with SSP2; Group 4, controls, received plasmid 1020 intramuscularly. Three vaccinations, approximately one month apart, were accomplished. Although the original protocol called for sporozoite challenge (20,000 sporozoites each of the St. Lucia strain of P. falciparum) three weeks after the last vaccination, low antibody titers pre-cluded following the protocol. A fourth vaccination, approximately 16 weeks after the third vaccination has been accomplished. Sporozoite challenge is planned at 3 weeks post vaccination and all animals are scheduled to be splenectomized 14 days post challenge.

Three monkeys have been excluded from the study: one, pregnant, from Group 2; one, with baby, from Group 3; and one, from Group 4, died of intercurrent infection.

G. Infectivity of the 3D7 clone sporozoites of Plasmodium falciparum from Panamanian Aotus

A reliable source of sporozoites is essential to ascertain the effectiveness of pre-erythrocytic vaccines. Monkeys vaccinated with the first such vaccine are awaiting to be challenged with Santa Lucia strain P. falciparum sporozoites from mosquitoes fed on monkeys bearing this strain. Such a model has been proven to be a sporadic source of sporozoites. The 3D7 clone of the NFS4 strain of P. falciparum, grown in vitro, produces abundant gametocytes for infecting mosquitoes by the membrane feeding technique, producing an almost on demand supply of sporozoites.

To determine if 3D7 sporozoites will produce patent infections in Panamanian Aotus, each of four normal Aotus was inoculated intravenously with 2.7×10^6 sporozoites from Anopheles stephensi mosquitoes. All monkeys were splenectomized on day 6 post inoculation. To date, 20 days post inoculation, no parasites have been detected on blood films. If a patent infection occurs, attempts will be made to adapt the 3D7 clone to Panamanian Aotus.

H. Production of interferon in Aotus by vaccination with Interleukin-12

Results of an experiment at another laboratory showed that when rhesus monkeys (Macaca mulatta) received rHuiL-12 on each of two days prior to inoculation with P. knowlesi sporozoites, the animals were completely protected against infection. A pilot experiment was carried out to determine if IL-12, a human agent, would stimulate gamma interferon in Aotus, as determined by serum biossay. If such stimulation occurred, an experiment was planned to ascertain if Aotus vaccinated with IL-12 would be protected when challenged with P. falciparum sporozoites.

Four Aotus, cured of P. falciparum and P. vivax infections were divided into two groups of two animals each. Group 1. Animals were vaccinated subcutaneously with 10 µg/kg of IL-12, while animals in Group 2 received 1.0 ml of 1% normal monkey serum/phosphate buffer saline. All animals were vaccinated on two consecutive days, and serum obtained

approximately 72 hours after the last injection. Bioassay results indicated that gamma interferon was not stimulated in either animal in Group 1.

I. Optimization of antibody responses of a malaria vaccine in Aotus

As previously reported, Aotus, cured of P. falciparum and P. vivax infections, were immunized with a DNA malaria vaccine, Py CSP intramuscularly, at various doses, with and without muscle pre-treatment, intradermally, at weeks 0, 3, and 6. Only monkeys injected intradermally produced significant antibody levels against sporozoites as measured by both the Immunofluorescent Assay Test (IFAT) and Enzyme-linked Immunosorbent Assay. Following three immunizations, antibody titers in the groups vaccinated intradermally peaked briefly at week 9, but declined to 50% of their peak values by week 14. There was a general trend towards a dose response in these monkeys. By week 46, anti - Py CSP antibody titers declined to 20%, 2%, and 6% of the week 14 peak values for the 2000, 500, and 125µg doses, respectively. At week 47, 16 monkeys received a fourth intramuscular dose of vaccine, 8 muscle pretreated; 7 monkeys received a fourth intradermal dose. At week 49, anti - Py CSP antibody titers in the intradermally immunized groups had geometric IFAT titers of 28, 963, 10, 240, and 6,451 for the 2000, 500, and 125 µg doses of plasmid DNA, respectively. These antibody titers were equivalent to titers generated with a Py CSP multiple Ag peptide (MAP) vaccine delivered with an adjuvant. No significant antibody titers were detected after the fourth dose in the intramuscularly immunized groups.

III. Conclusions

Since folinic acid co-administered with WR 227825 (1.0 mg/kg x 3 days) had an adverse synergistic effect, an experiment is **planned to evaluate WR 227825, alone, at a dose of 0.1 mg/kg (x 3 days)** against Vietnam Smith/RE strain infections of P. falciparum.

While halofantrine (WR 171669) at an initial treatment dose of 5.0 mg/kg, cleared, but did not cure infections, its metabolite, desbutylhalofantrine (WR 178460), did not clear parasitemias with a primary treatment of 5.0 mg/kg. Both drugs (20.0 mg/kg) administered at a primary treatment, cleared, but without cure. Repeat treatments at higher doses with halofantrine cured a total of 6 of 8 infections (75%); repeat

treatments with its metabolite cured a total of 4 of 9 infections (44%). Neither drug shows significant curative activity against Vietnam-Oak Knoll strain infections of P. falciparum.

Results of establishing a sporozoite-induced infection model with the Santa Lucia strain of P. falciparum in Panamanian Aotus indicate that splenectomy on day 7 post-inoculation produces the greatest number of patent infections (75%). Future experiments using this model to evaluate a DNA pre-erytrocytic vaccine will incorporate splenectomy on day 7 post inoculation.

The first evaluation of a DNA erythrocytic vaccine (MSP-1) showed that the monkeys were not protected against challenge of P. falciparum (Vietnam-Oak Knoll strain). It is anticipated that other DNA erythrocytic vaccines will be evaluated.

Homologous re-challenge with Vietnam-Oak Knoll parasites has, to date, resulted in four Aotus with sterile immunity. These animals, as well as others without such immunity will be re-challenged both with homologous parasites, and eventually a heterologous strain. Data will be compared with a hopefully effective DNA vaccine.

It is anticipated that the Aotus immunized with a pre-erythrocytic vaccine will be challenged with Santa Lucia sporozoites within 30-60 days.

To date, the absence of patent infections following sporozoite inoculation of the 3D7 clone of the NF54 strain of P. falciparum would appear to indicate that the clone would not serve as a replacement for Santa Lucia sporozoites. No further experiments are planned to use sporozoites of the 3D7 clone.

The failure of rHu-IL-12 to stimulate gamma interferon in Aotus precludes further trials.

The significantly high antibody titers following the fourth intradermal immunization of Aotus with a Py CSP plasmid DNA based vaccine were equivalent to an optimal MAP/adjuvant based vaccine and support the use of the intradermal route for studies on the efficacy of DNA vaccine in inducing protective antibodies against P. falciparum.

REFERENCES

1. Schmidt, LH. 1978. Plasmodium falciparum and Plasmodium vivax infections in the owl monkey (Aotus trivirgatus). I. The courses of untreated infections. Am J Trop Med Hyg. 27:671-702.

2. Schmidt, LH 1978. Plasmodium falciparum and Plasmodium vivax infections in the owl monkey (Aotus Trivirgatus). II. Responses to chloroquine, quinine, and pyrimethamine. Am J Trop Med Hyg. 27:703-717.

3. Rossan, RN, Harper, JS III, Davidson, DE Jr., Escajadillo, A. and Christensen, HA. 1985. Comparison of Plasmodium falciparum infections in Panamanian and Colombian owl monkeys. Am J Trop Med Hyg. 34:1037-1047.

4. Davidson, DE Jr., Ager, AL, Brown, JL, Chapple, FE, Whitmire, RE, Rossan, RN. 1981. New tissue schizontocidal antimalarial drugs. Bull WHO. 59:463-479.

5. Milhous, WK, Shuster, BG, Theoharrides, AD, Davidson, DE Jr., Heisey, GE, Ward, G, Dutta, PK, Puri, SK, Dhar, MM, Rossan, RN. New Alternatives to primaquine. Presented at XII International Congress forTropical Medicine and Malaria. Amsterdam.

6. Pollack, S., Rossan, RN, Davidson, DE, Escajadillo, A., 1987. Desferrioxamine suppresses Plasmodium falciparum in Aotus monkeys. Proc Soc Expt Biol Med. 184:162-164.

7. Panton, LJ, Rosssan, RN, Escajadillo, A, Matsumoto, T, Lee, AT, Labroo, VM, Kirk KL, Cohen, LA, Airkawa, M, Howard, RJ. 1988. In vitro and in vivo studies of the effects of halogenated histidine analogs on Plasmodium falciparum. Antimicrob Agents Chemoth. 32:1655-1659.

8. Martin, SK, Oduola, AMJ, Milhous, WK. 1987. Reversal of chloroquine resistance in Plasmodium falciparum by verapamil. Science. 235:899-901.

9. Krogstad, DJ, Gluzman, IY, Kyle, DE, Oduola, AMJ, Martin, SK, Milhous, WK, Schlesinger, PH. 1987. Efflux of chloroquine from Plasmodium falciparum: mechanism of chloroquine resistance. Science. 238:1283-1285.

REFERENCES (CONT'D)

10. Bitonti, AJ, Sjoerdsma, A, McCann, PP, Kyle, DE, Oduola, AMJ, Rossan, RN, Milhous, WK, Davidson, DE Jr. 1988. Reversal of cloroquine resistance in malaria parasite Plasmodium falciparum by desipramine. Science. 242:1301-1303.

11. Kyle, DE, Milhous, WK, Rossan, RN. 1993. Reversal of Plasmodium falciparum resistance to chloroquine in Panamanian Aotus monkeys. Am J Trop Med Hyg. 48:126-133.

12. Inselburg J, Bzik DJ, Li W, Green KM, Kansopon J, Hahm BK, Bathurst IC, Barr PJ, Rossan RN. 1991. Protective immunity induced in Aotus monkeys by recombinant SERA proteins of Plasmodium falciparum. Inf. Imm. 59:1247-1250.

13. Inselburg J, Bathurst IC, Kansopon J, Barchfeld GL, Barr PJ, Rossan RN. 1993. Protective immunity induced in Aotus monkeys by a recombinant SERA protein of Plasmodium falciparum: Adjuvant effects on induction of immunity. Inf. Imm. 61:2041-2047.

14. Inselburg J, Bathurst IC, Kansopon J, Barr PJ, Rossan RN. 1993. Protective immunity induced in Aotus monkeys by a recombinant SERA protein of Plasmodium falciparum: Further studies using SERA 1 and MF75.2 adjuvant. Inf Imm. 61:2048-2052.

15. Earle, EC and Perez, M. 1931. Enumeration of parasites in the blood of malarial patients. J Lab Clin Med. 19:1124-1130.

16. Schmidt, LH, Crosby, R, Rasco, J, Vaughn, D. 1978. Antimalarial activities of various 9-phenanthrenemethanols with special attention to WR-122,455 and WR-171,699. Antimicrob Agents Chemotherapy. 14:292-314.

TABLE 1

DETAILED ACTIVITY OF WR 171669AU (BM 01792) AGAINST VIETNAM - OAK-KNOLL STRAIN INFECTIONS OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12754	5.0	1	2	1	<0.01	<0.01	0	0	0	0	0	0
12755	5.0	2	19	0.7	<0.01	<0.01	0	0	0	0	0	0
12775	5.0	0.5	3	0.6	<0.01	<0.01	0	0	0	0	0	0
12760	20.0	1	9	0.1	<0.01	<0.01	<0.01	0	0	0	0	0
12773	20.0	0.9	5	0.3	<0.01	<0.01	<0.01	0	0	0	0	0
12774	20.0	0.5	3	0.6	<0.01	<0.01	0	0	0	0	0	0
12754r	20.0	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	0
12755r	20.0	<0.01	49	1	<0.01	<0.01	0	0	0	0	0	0
12775r	20.0	3	2	1	0.3	<0.01	0	0	0	0	0	0
12760r	45.0	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	0
12773r	45.0	0.9	4	9	1	<0.01	0	0	0	0	0	0
12774r	45.0	0.9	23	0.6	0.01	<0.01	0	0	0	0	0	0
12755rr	45.0	<0.01	0.2	<0.01	0	0	0	0	0	0	0	0
12773rr	90.0	0.01	0.01	0	0	0	0	0	0	0	0	0

TABLE 2

SUMMARY OF THE ACTIVITY OF WR 171669AU (BM 01792) AGAINST VIETNAM OAK KNOLL STRAIN INFECTIONS OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12754	5.0			+	5	10	Re-Rx, higher dose
12755	5.0			+	5	8	Re-Rx, higher dose
12775	5.0			+	5	16	Re-Rx, higher dose
12760	20.0			+	6	11	Re-Rx, higher dose
12773	20.0			+	6	26	Re-Rx, higher dose
12774	20.0			+	5	8	Re-Rx, higher dose
12754r	20.0			+	3	n.a.	Cured
12755r	20.0			+	5	20	Re-Rx, higher dose
12775r	20.0			+	4	n.a.	Cured
12760r	45.0			+	3	n.a.	Cured
12773r	45.0			+	5	17	Re-Rx
12774r	45.0			+	4	n.a.	Cured
12755rr	45.0			+	3	n.a.	Cured
12773rr	90.0			+	2	n.a.	Cured

TABLE 3

DETAILED ACTIVITY OF WR 178460AC (BM 08577) AGAINST
VIETNAM OAK-KNOLL STRAIN INFECTIONS OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12781	5.0	0.6	0.9	2	0.4	0.1	<0.01	<0.01	<0.01	<0.01	<0.01	0.4
12782	5.0	0.5	0.8	6	10	9	46	25	63	Re-Rx, higher dose		
12784	5.0	0.9	2	10	10	8	26	28	216	Re-Rx, higher dose		
12778	20.0	0.9	4	0.8	<0.01	<0.01	0	0	0	0	0	0
12783	20.0	0.7	2	1	<0.01	<0.01	0	0	0	0	0	0
12785	20.0	0.6	2	1	<0.01	<0.01	0	0	0	0	0	0
12781r	20.0	160	120	26	<0.01	<0.01	<0.01	0	0	0	0	0
12782r	20.0	63	72	20	0.6	<0.01	<0.01	0	0	0	0	0
12784r	20.0	216	222	161	13	16	0.6	<0.01	0	0	0	0
12778r	45.0	<0.01	<0.01	<0.01	0	0	0	0	0	0	0	0
12783r	45.0	<0.01	43	<0.01	<0.01	<0.01	0	0	0	0	0	0
12785r	45.0	0.3	83	<0.01	<0.01	<0.01	0	0	0	0	0	0
12782rr	45.0	<0.01	4	<0.01	<0.01	0	0	0	0	0	0	0
12783rr	90.0	1	20	1	<0.01	0	0	0	0	0	0	0
12778rr	90.0	1	37	20	0.5	<0.01	0	0	0	0	0	0

TABLE 4

SUMMARY OF THE ACTIVITY OF WR 178460AC (BM 08577) AGAINST VIETNAM OAK-KNOLL STRAIN INFECTIONS OF PLASMODIUM FALCIPARUM

Monkey No.	Daily Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12781	5.0		+		n.a.	n.a.	Re-Rx, higher dose
12782	5.0		+		n.a.	n.a.	Re-Rx, higher dose
12784	5.0		+		n.a.	n.a.	Re-Rx, higher dose
12778	20.0			+	5	9	Re-Rx, higher dose
12783	20.0			+	5	8	Re-Rx, higher dose
12785	20.0			+	5	9	Re-Rx, higher dose
12781r	20.0			+	6	n.a.	Cured
12782r	20.0			+	6	31	Re-Rx, higher dose
12784r	20.0			+	7	n.a.	Cured
12778r	45.0			+	3	17	Re-Rx, higher dose
12783r	45.0			+	5	19	Re-Rx, higher dose
12785r	45.0			+	5	n.a.	Cured
12782rr	45.0			+	4	n.a.	Cured
12783rr	90.0			+	4	102	
12778rr	90.0			+	5	54	

TABLE 5

SUMMARY OF THE ACTIVITIES AGAINST
PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
Vietnam Oak Knoll	WR 171669AU (BM 01792) - halofantrine							
	15.0	5.0	3/3	0/3			3/3	0/3
	60.0	20.0	3/3	0/3	3/3	2/3	6/6	2/6
	135.0	45.0			4/4	3/4	4/4	3/4
	270.0	90.0			1/1	1/1	1/1	1/1
	WR 178460AC (BM 08577) - desbutylhalofantrine							
	15.0	5.0	0/3	0/3			0/3	0/3
	60.0	20.0	3/3	0/3	3/3	2/3	6/6	2/6
	135.0	45.0			4/4	2/4	4/4	2/4
	270.0	90.0			2/2	0/2	2/2	0/2

TABLE 6

SPOROZOITE-INDUCED INFECTIONS OF THE

SANTA LUCIA STRAIN OF PLASMODIUM FALCIPARUM

IN AOTUS L. LEMURINUS

MONK. NO.	PREPATENT PD. (DAYS)	MAXIMUM PARASITEMIA PER CMM (x 10^3)	RECRUD-ESCENCE
	GROUP 1 - Splenectomized prior to inoculation		
12733	23	357	0
12734	21	434	3
12736	0		
12737	0		
	GROUP 2 - Splenectomized day 7 post inoculation		
12716	21	494	(a)
12741	29 (one day only)		(b)
12743	23	616	1(c)
12753	29	296	4
	GROUP 3 - Splenectomized day 37 post inoculation		
12746	0		
12747	23	154	2
12750	0		
12751	39	611	(d)

(a) Died day 79 post-inoculation, malaria.
(b) Died day 114 post-inoculation, cardiac clot.
(c) Died day 91 post-inoculation, intestinal haemorrhage, fatty liver
(d) Died day 76 post-inoculation, malaria.

TABLE 7

CHALLENGE WITH THE FVO STRAIN OF PLASMODIUM FALCIPARUM

MONK NO.	NO. OF CHALLENGES	NOTES
12727	4	Sterile immunity
12730	3	Sterile immunity
12735	4	Sterile immunity
12739	3	Parasitemias of <10
12748	1	Treatment required
12749	2	Sterile immunity
12752	1	Not immune
12756	1	Not immune
12757	1	Not immune
12759	1	Not immune
12762	1	Not immune
12763	1	Not immune
12764	1	Not immune
12765	1	Not immune
12169	1	Died day 32 post-challenge, malaria
12687	1	Rx,died day 46 post-challenge, inter-current infection
12738	1	Died day 19 post-challenge, malaria
12740	1	Rx,died day 51 post challenge inter-current infection

DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 7 Feb 97

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCP, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Contract Number DAMD17-91-C-1072. Request the limited distribution statement for Accession Document Numbers ADB214740, ADB198405, ADB210896, ADB183789, and ADB173254 be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Mrs. Judy Pawlus at DSN 343-7322.

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GARY R. GILBERT
Colonel, MS
Deputy Chief of Staff for
Information Management